PB83-163469

Metabolism and DNA (Deoxyribonucleic Acid) Binding of Benzo(a)pyrene in Cultured Human Bladder and Bronchus

Medical Coll. of Ohio at Toledo

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

30 Nov 82

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before comple

1. REPORT NO. EPA-600/J-82-274	2. JOURNAL ARTICLE	3. PB83-163469
4. TITLE AND SUBTITLE Journal Article: Metabolism and DNA binding of benzo[a]pyrene in cultured human bladder and bronchus		5. REPORT DATE 11/30/82 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) G.D.Stoner, F.B.Daniel, K.M.Schenck, H.A.J.Schut, P.J.Goldblatt, and D.W.Sandwisch		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Health Effects Research Laboratory, U.S.E.P.A., 26 W. St. Clair St. Cincinnati, OH. 45268, AND Department of Pathology, Medical College of Ohio, 3000 Arlington Ave. Toledo, OH. 43614.		10. PROGRAM ELEMENT NO. CCSLIA 11. CONTRACT/GRANT NO. CR 807670
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research & Development Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED Part 1 of final 8/80-8/83 14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES Carcinogenesis, Vol. 3, No. 2, pp. 195-201, 1982.
Project Officer: F. B. Daniel, TMD, HERL. This is part of the final report of this project.

16. ABSTRACT

The metabolism of benzo[a]pyrene (BP) was examined in explant cultures of human bladder and bronchus. Three-day cultures were exposed to radiolabeled BP for 24 h, and the metabolism was determined by analysis of the level of binding of reactive metabolites to DNA, and by the release of metabolites into the medium. For a given individual, the DNA binding level and extent of metabolism was usually higher in the bladder than in the bronchus. In specimens obtained from 16 individuals, the average DNA-binding levels for BP–DNA adducts following a 24 h exposure to 1 μM BP were 6.4 ± 5.0 μmol BP/mol deoxyribonucleotide for the bladder and 3.1 ± 1.9 μmol BP/mol deoxyribonucleotide for the bronchus. The major BP–DNA adduct in both tissues co-chromatographed with one of the adducts formed by reaction of *r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo[a]pyrene with deoxyguanosine using high-pressure liquid chromatography. In tissues obtained from the same individual, the binding levels of BP metabolites to bladder cell DNA was not strongly correlated to that of bronchial cell DNA (r = 0.54). The medium of both tissues contained small amounts of free, unconjugated metabolites of BP (<3% of the total) and large amounts (30–85% of the total) of unidentified, highly polar material. Human bladder appears to be the most active human explant tissue yet studied with respect to its ability to activate BP to DNA binding forms. The relevance of this observation to human bladder cancer is, as yet, unknown.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Carcinogen-DNA Adducts, Benzo(a)pyrene,		

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 8
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

Carcinogenesis, Vol 3 No. 2, pp.195–201, 1982
JOURNAL ARTICLE:

EPA-600/J-82-274
JOURNAL ARTICLE

Metabolism and DNA binding of benzo[a]pyrene in cultured human bladder and bronchus

Gary D. Stoner[1], F. Bernard Daniel[2], Kathleen M. Schenck[2], Herman A.J. Schut[1], Peter J. Goldblatt[1] and David W. Sandwisch[1]

[1]Department of Pathology, Medical College of Ohio, 3000 Arlington Avenue, Toledo, OH 43614, and [2]Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH 45268, USA.

(Received on 1 October 1981; revised on 23 November 1981; accepted on 5 December 1981)

Abstract

The metabolism of benzo[a]pyrene (BP) was examined in explant cultures of human bladder and bronchus. Three-day cultures were exposed to radiolabeled BP for 24 h, and the metabolism was determined by analysis of the level of binding of reactive metabolites to DNA, and by the release of metabolites into the medium. For a given individual, the DNA binding level and extent of metabolism was usually higher in the bladder than in the bronchus. In specimens obtained from 16 individuals, the average DNA-binding levels for BP – DNA adducts following a 24 h exposure to 1 μM BP were 6.4 ± 5.0 μmol BP/mol deoxyribonucleotide for the bladder and 3.1 ± 1.9 μmol BP/mol deoxyribonucleotide for the bronchus. The major BP – DNA adduct in both tissues co-chromatographed with one of the adducts formed by reaction of *r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo[a]pyrene with deoxyguanosine using high-pressure liquid chromatography. In tissues obtained from the same individual, the binding levels of BP metabolites to bladder cell DNA was not strongly correlated to that of bronchial cell DNA (r = 0.54). The medium of both tissues contained small amounts of free, unconjugated metabolites of BP (<3% of the total) and large amounts (30–85% of the total) of unidentified, highly polar material. Human bladder appears to be the most active human explant tissue yet studied with respect to its ability to activate BP to DNA binding forms. The relevance of this observation to human bladder cancer is, as yet, unknown.

Introduction

The majority of human cancers arise in the epithelium and are thought to be due to exposure to environmental chemical carcinogens (1). Recent developments in the maintenance and growth of viable human epithelial tissues and cells *in vitro* have made it possible to study chemical carcinogenesis directly in human tissues. Initial studies have shown that cultured tissues and whole cells from several human organs including bronchus (2–8), colon (9–11), lung (7,12,13), esophagus (14), pancreatic duct (6), mammary gland (15), endometrium (16), skin (17) and blood monocytes (18) are capable of metabolizing chemical carcinogens.

The metabolism of the environmental procarcinogen, benzo[a]pyrene (BP)*, has been studied extensively in cultured human tissues. BP was converted by human tissues into forms that were bound to cellular DNA (2,3,6,7,11,13,14,16) and protein (7,9,13,14) and, there was a marked variation in the ability of tissues from different individuals to metabolize BP (3,7,9,11,14). A 75-fold, 100-fold, 99-fold, and 9-fold variation between individuals was found in the binding of BP to bronchial cell DNA (3), to colonic cell DNA (9,11), to esophageal cell DNA (14) and to alveolar macrophage DNA (7), respectively. The adducts formed between the metabolites (generally, diol epoxides) of BP and DNA in cultured human bronchus (19), colon (11), endometrium (16), and esophagus (14) have been identified. The metabolism of BP in cultured human respiratory tissues was qualitatively similar to that of respiratory tissues from various animal species in which BP has been shown experimentally to be carcinogenic (8).

In the present report, we describe the metabolism of BP in cultured explants of human bronchus and bladder. The comparison of BP metabolism in these tissues was undertaken because cigarette smoking is epidemiologically linked to cancer of the human lung (20) and bladder (21), and cigarette smoke contains BP (22). The data indicate that: (a) the pathways for the metabolic activation of BP in cultured human bladder are similar to those in the bronchus, (b) in tissues taken from the same patient, the DNA binding levels of BP metabolites are usually higher in the bladder than in the bronchus, (c) there are significant variations among individuals in the extents of binding of BP metabolites to both bronchus and bladder DNA, and (d) the adducts formed between BP and DNA in both tissues are identical. A preliminary report of this study has been presented (23).

Materials and Methods

Chemicals

BP (generally tritium-labeled; 30–40 Ci/mmol) was purchased from Amersham Corporation, Arlington Heights, IL, and diluted to a specific radioactivity (SA) of 3–5 Ci/mmol with unlabeled BP (Eastman Organic Chemicals, Rochester, NY). The compounds were purified and judged to be chemically and radiochemically pure by h.p.l.c. The SA was calculated from the molar absorptivity of BP (ϵ_{295}^{MeOH} = 60 000) and determination of radioactivity by liquid scintillation counting. Purified [^{3}H]BP was dissolved in a mixture of dimethylsulfoxide (DMSO): methanol (4:1) and stored at 4°C at a concentration of 0.1 mM before addition to the explant cultures.

Authentic metabolites of BP were obtained from the Chemical Repository of the National Cancer Institute, Bethesda, MD. All compounds were stored at −20°C in the dark. Hydroxylapatite (HTP-DNA grade) was purchased from Bio-Rad, Richmond, CA and stored at 4°C until used. All solvents for chromatography were h.p.l.c. grade and obtained from Fisher Scientific, Pittsburgh, PA. DMSO was purchased from Burdick-Jackson, Muskegon, MI.

Tissue specimens

Grossly normal bladder and bronchial tissues were taken from 16 patients at autopsy within 2–6 h of the time of death. The age, sex, and time after the patient's death, before tissues were placed in culture are given in Table I. Tissues from patients with sepsis, metastatic cancer, serum hepatitis or a history of tuberculosis were not used in these studies. The tissues were immersed in ice-cold L-15 medium immediately after removal from the patient

*Abbreviations: BP, benzo[a]pyrene; SA, specific radioactivity; (±) *anti* diol-epoxide, *r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo[a]pyrene; (±) *syn* diol-epoxide, *r*-7,*t*-8-dihydroxy-*c*-9,10-oxy-7,8,9,10-tetrahydrobenzo[a]prene; dG, deoxyguanosine; ODS, octadecylsilane; BP-9,10-DHD, *trans*-9,10-dihydro-9,10-dihydroxybenzo[a]pyrene; BP-4,5-DHD, *trans*-4,5-dihydro-4,5-dihydroxybenzo[a]pyrene; BP-7,8-DHD, *trans*-7,8-dihydro-7,8-dihydroxybenzo[a]pyrene; BP-9-ol, 9-hydroxybenzo[a]pyrene; BP-3-ol, 3-hydroxybenzo[a]pyrene.

IRL Press Ltd., Oxford, England. 0143-3334/82/0302-0195 $2.00/0

and transported to the laboratory. Using aseptic conditions, muscle and connective tissues were removed from the bladder specimens as thoroughly as possible leaving the epithelial layer intact. The bronchial specimens were trimmed of adherent lung tissues. Pieces of tissue from each specimen were fixed in a phosphate buffered solution containing 4% formaldehyde and 1% glutaraldehyde (4F1G) (24), embedded in Epon and assessed by light microscopy after staining with toluidine blue.

Explant culture conditions

Bladder and bronchial samples were cut into ~0.5 x 0.5 cm pieces and placed on the etched surface of 60 mm plastic tissue culture dishes (one piece/dish) with the epithelium oriented toward the gas-liquid interface (25). The number of bladder pieces obtained from each case ranged from 4 to 28, and the number of bronchial pieces from 4 to 78. To each culture dish was added 2.5 ml of PFMR-4 medium (26) supplemented with crystalline bovine insulin (1 μg/ml, Eli Lilly, Indianapolis, IN), hydrocortisone hemisuccinate (35 ng/ml, Upjohn Co., Kalamazoo, MI), β-retinyl acetate (0.1 μg/ml, Hoffmann LaRoche, Nutley, NJ), gentamicin (50 μg/ml), penicillin G (100 U/ml), kanamycin (100 μg/ml), and amphotericin B (2.5 μg/ml). The cultures were maintained in an air-tight chamber at 36.5°C in an atmosphere of 50% O_2 – 45% N_2 – 5% CO_2 at a pressure of 3 psi. The chamber was placed on a rocker platform (Bellco Glass Co., Vineland, NJ) and rocked in the dark at ~5 cycles/min so that the tissues were alternately exposed to the medium and to the atmosphere. Every 2 days the medium was replaced and the atmosphere of the cultures was restored by flushing the chamber with the gas mixture at 5 l/min for 5 min.

Incubation with [^{3}H]BP and sample preparation

On the third day of explant culture [^{3}H]BP (3 – 5 Ci/mmol) was added to the culture medium at a concentration of 1 μM. After incubation with carcinogen for 24 h, the medium and tissues were separated and the media were stored immediately at −20°C. The explants were rinsed twice with cold phosphate buffered saline (PBS) containing 0.1% glucose. Bladder explants were then pooled and stored at −20°C. The bronchial mucosa containing epithelial and mesenchymal cells was stripped from the supporting structures with forceps and stored at −20°C. Cells from 4 to 8 cultures were pooled for each experimental variable. Incubations of medium with [^{3}H]BP but without the explants served as controls.

Preparation of BP – DNA adduct standards

Synthetic BP – DNA adducts were prepared by a method similar to that of Remsen, *et al.* (27). Briefly, 0.95 mg of calf thymus DNA (P & L Biochemicals, Milwaukee, WI) was dissolved in 1 ml of water and treated with 0.4 ml of methanol followed by addition of 93 μl of a solution of racemic *r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo[a]pyrene [(±) *anti* diol-epoxide] in DMSO (7 mg/ml). The reaction mixture was incubated at 37°C for 3.5 h, followed by addition of 150 μl of saturated NaCl and precipitation of the DNA by addition of one volume of 2-ethoxyethanol. The BP-modified DNA was washed twice with absolute ethanol, and twice with anhydrous ether and stored at −20°C until used. A portion of this material was hydrolyzed by enzymatic procedures (see below) and extracted with 1 volume of water-saturated *n*-butanol. Following removal of the *n*-butanol under N_2 the residue was dissolved in 50% methanol in H_2O and chromatographed as described below. The major u.v. peaks eluting at 63% (peak fraction 27) and 65% (peak fraction 31) methanol were collected and their u.v.-spectra were identical to those reported by Jeffrey, *et al.* (19) for BP – DNA adducts; i.e. 7S-*anti*-diol-epoxide-dG (peak fraction 27) and 7R-*anti* diol-epoxide-dG (peak fraction 65). These adducts were dissolved in methanol, stored at −20°C, and used as chromatographic standards.

Isolation of [^{3}H]BP bound to DNA

Bladder and bronchial explants were homogenized in 2 ml 0.05 M sodium phosphate buffer, pH 6.5, containing 0.01 M EDTA and 0.01 M EGTA, using a Tissumizer (Tekmar, Cincinnati, OH) at maximum speed for 30 s. The homogenates were made 1.5% (v/v) in sodium dodecyl sulfate, treated with 0.2 mg autodigested pronase B, (Calbiochem, LaJolla, CA) and incubated for 2.5 h at 37°C with mixing. The samples were made 7% (w/v) in 4-aminosalicylate-NaCl (6:1) and extracted for 1 h with 1 volume of phenol reagent (28), after which the phases were separated by centrifugation (40 min at 12,000 x g) and the aqueous phase removed. The phenol phase was back-extracted with 1 ml buffer, and the resulting aqueous phases pooled. Hydroxylapatite (0.5 g), suspended in 2 ml 0.05 M sodium phosphate buffer, pH 6.5, was added to the pooled aqueous layers in a 15 ml polystyrene centrifuge tube. The gel was washed four times with 10 ml volumes of a solution of 8 M urea in 0.12 M sodium phosphate buffer, pH 6.5, in a batch procedure, followed by two 5 ml washes of 0.05 M sodium phosphate buffer. The DNA was then eluted from the hydroxylapatite with two 1.5 ml washes of 0.55 M sodium phosphate buffer, pH 6.5. The amount of DNA present in the aqueous samples was determined fluorimetrically after reaction of an aliquot (150 μl) with 3,5-diaminobenzoic acid (29). The radioactivity associated with the DNA was determined by liquid scintillation counting in PCS (Amersham, Arlington Heights, IL) after digestion with perchloric acid. The binding levels were then calculated and expressed as μmol BP/mol deoxyribonucleotide.

BP-DNA adduct chromatography

The purified BP-modified DNA solutions were pooled, dialyzed, dried and then redissolved in 2 ml 0.1 M Tris buffer, pH 7, containing 10 mM $MgCl_2$, 1 mM $ZnCl_2$. The DNA was hydrolyzed enzymatically to the nucleoside level with DNase I, snake venom phosphodiesterase, and alkaline phosphatase (Worthington, Bedford, MA) at 37°C for 16 h. Samples were checked by t.l.c. on cellulose (Eastman No. 13254) with 1 M ammonium acetate, pH 7.5: ethanol (33:67) against deoxyribonucleoside and deoxyribonucleotide standards to ensure that hydrolysis to the nucleoside level was complete. The BP – DNA adducts were extracted from the hydrolysate into an equal volume of *n*-butanol (water saturated), the butanol layer removed, and dried under N_2 at 37°C. The resulting residue was redissolved in 1 ml 50% MeOH in H_2O for h.p.l.c.

The [^{3}H]BP – DNA adducts from bladder and bronchial tissues and synthetic adduct standards were co-chromatographed on a Varian model 5000 instrument programmed with a linear methanol in water gradient of 50 to 80% over 30 min on a Zorbax-ODS column (4.6 x 250 mm). A flow rate of 1 ml/min was maintained throughout the run and 0.5 ml fractions were collected with an ISCO model 1800 fraction collector fitted with a flow interruptor (ISCO model 590). The effluent was monitored at 330 nm with a Varian Vari-chrom UV detector and for fluorescence (excitation = 330 nm; emission >389 nm) with a Schoeffel model GM 970 fluorescence detector. To each collected fraction, 5 ml of PCS (Amersham, Arlington Heights, IL) was added and all samples were counted in a LKB model 1216 liquid scintillation counter equipped with a DPM-PLOT package.

Analysis of BP metabolites in culture medium

H.p.l.c. analysis of BP metabolites released into the medium by cultured explants of bladder and bronchus was carried out on a LDC (Riveria Beach, FL) gradient system driven by a LDC computer control module (CCM). A 4.6 x 150 mm ODS column (LDC 5 μm Spherisorb) was eluted with 10 to 100% methanol in water gradients. The effluent was monitored for u.v. absorbance with a LDC Spectromonitor III detector and radioactivity with a Radiomatic Instruments (Miami, FL) FLO-ONE detector. The tracings from these two detectors were recorded in real time and integrated by the CCM. Sixty fractions of 1 ml were collected in 7 ml mini-vials with an ISCO (Lincoln, NB) model 1800 fraction collector fitted with an ISCO model 690 flow interrupter valve.

Radioactive material in the medium was analyzed by mixing 0.9 ml of media with 0.1 ml of methanol and injecting the mixture via a 1 ml sample loop onto the column. The fractions were mixed with 4 ml of PCS, and the radioactivity was determined.

Results

Prior to the initiation of these experiments, it was necessary to determine the period of time after death that bladder and bronchial tissues could be obtained and maintained as viable explants in culture. Therefore, using histological techniques, the morphology of specimens of bladder and bronchus taken from 24 patients at autopsy was assessed prior to culture and after 1, 2, 3, 5, 7, 10, 14, 21 and 28 days of incubation. In addition, pieces of both tissues from each patient were placed in the center of 60 mm tissue culture dishes and incubated for 14 days to determine if epithelial cells would grow out from the periphery of the tissues onto the culture dishes (30,31). Results from these experiments indicated that bladder and bronchial tissues obtained within 6 h of death could, in some cases, survive for 28 days in rocking explant culture, and exhibited epithelial cell outgrowths in primary culture. However, some variation with respect to these parameters was observed amongst patients. The morphology of bladder and bronchial explants maintained for 7 days in rocking organ culture are given in Figures 1 and 2. Bladder and bronchial specimens collected from patients as long as 12 h after death were usually non-viable.

The level of [^{3}H]BP covalently bound to the DNA of both bronchus and bladder increased with time for a period of at

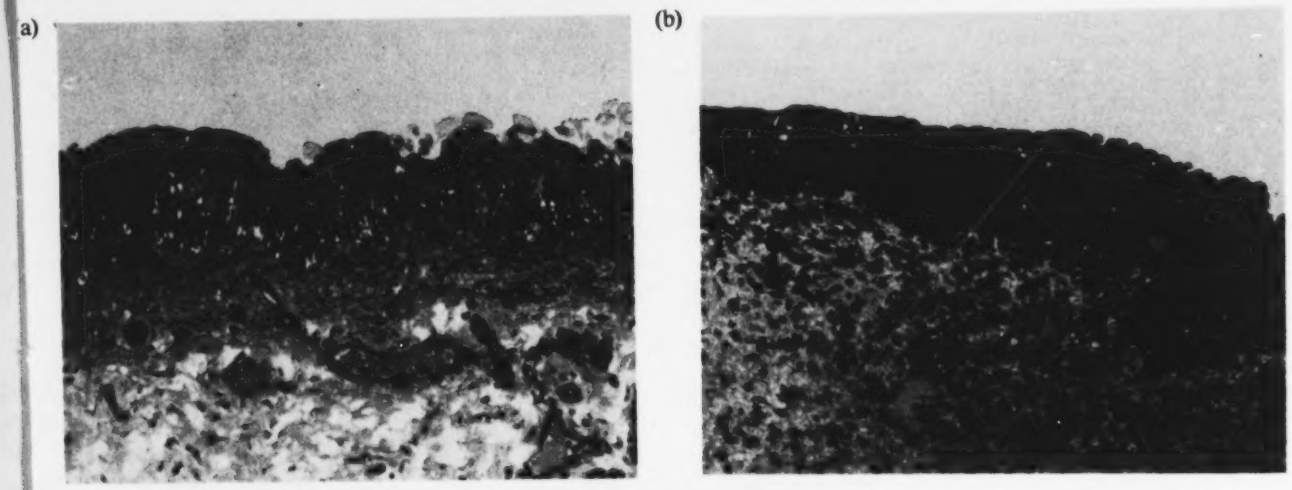

Fig. 1. (a) Specimen of human bladder prior to culture. x 400. (b) Explant of human bladder maintained for 7 days in rocking organ culture. x 400. Note preservation of explant morphology.

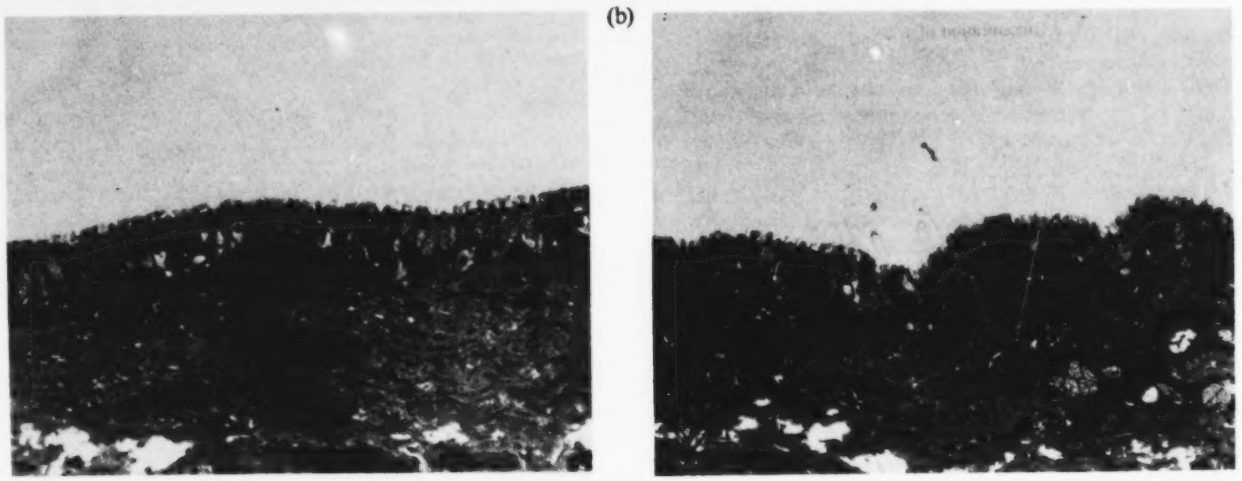

Fig. 2. (a) Specimen of human bronchus prior to culture. x 200. (b) Explant of human bronchus maintained for 7 days in rocking organ culture. x 200. Note preservation of normal mucociliary epithelium.

least 24 h (Figure 3). This figure also illustrates the higher level of binding of BP metabolites to bladder cell DNA than to bronchial cell DNA in explants cultured from the same patient. In separate experiments, we observed that the levels of BP metabolites bound to bladder and bronchial DNA increased with the concentration of [^{3}H]BP in the medium up to a dose of 10 μM, the highest concentration used.

Table I illustrates the variation among individuals in the levels of binding of BP metabolites to explant DNA in human bladder and bronchus. There was approximately a 34-fold interindividual variation in the levels of binding of BP metabolites to bladder cell DNA, while that to bronchial cell DNA was 10-fold. The levels of BP metabolites bound to the explant DNA were generally higher in the bladder (6.4 $\pm$ 5.0, μmol BP/mol deoxyribonucleotide, N = 16) than in the bronchus (3.1 $\pm$ 1.9 μmol BP/mol deoxyribonucleotide, N = 16). There was a weak positive correlation (r = 0.55) between the levels of BP bound to DNA in bladder and those in bronchial explants obtained from the same patient. Neither the bladder, nor the bronchus exhibited a correlation between time elapsed after death until culture of the tissue and extent of covalent binding ($r_{bladder} = -0.34$, $r_{bronchus} = -0.19$).

The h.p.l.c. profiles of the BP–DNA adducts formed in bronchus and bladder explants after a 24 h incubation with [^{3}H]BP are shown in Figure 4. In both tissues, the majority of the radioactivity associated with the DNA co-chromatographed with the chemical standards (arrows, Figure 4) previously shown to be adducts of the exocyclic amino group of deoxyguanosine (N^2) to the 10 carbon of the (±) *anti* diol-epoxide (19). In all cases studied, 80% or more of the total radioactivity associated with the purified DNA was accounted for in the major adduct, 7R-*anti* diol-epoxide. A smaller peak eluting ahead of these two standards was consistently observed in both human bronchus and bladder (Figure 4).

When the whole medium was analyzed by h.p.l.c., 2 major peaks of radioactive material were eluted in all cases: an early, polar peak (fractions 2–10) and a later peak (fractions

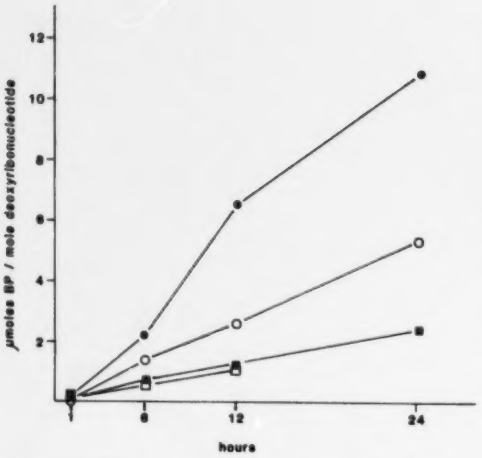

Fig 3. The level of [^{3}H]BP – DNA binding as a function of time of exposure to 1 μM [^{3}H]BP. Human bladder (case no. 98 = ○; case no. 100 = ●) and human bronchus (case no. 98 = □; case no. 100 = ■.

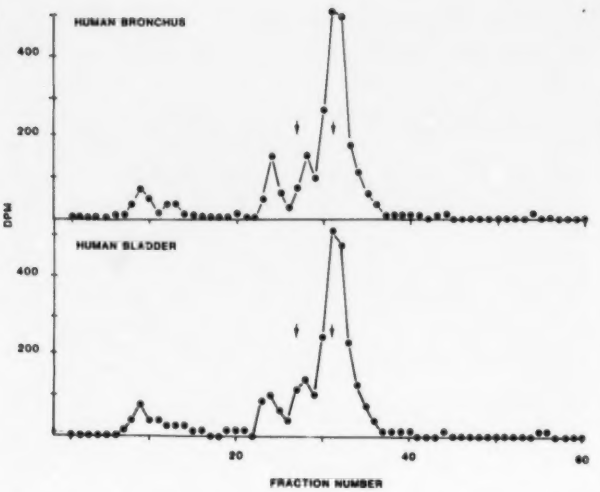

Fig. 4. H.p.l.c. profiles (Zorbax-ODS) of [^{3}H]BP – DNA adducts obtained from human bronchus (upper) and human bladder (lower) following incubation with 1 μM [^{3}H]BP. The arrows indicate the elution points of the two major adducts formed by the reaction of (±) *anti* diol-epoxide with calf thymus DNA, and represent 7S-*anti* diol-epoxide-dG (peak fraction 27) and 7R-*anti* diol-epoxide-dG (peak fraction 31).

49 – 51). Identified metabolites of BP (e.g. dihydrodiols, phenols, etc.) were eluted between these 2 peaks. The early, polar peak represented 30 – 85% of the total in case of the bladder and 35 – 83% of the total in case of the bronchus (Table I). The second peak of radioactive material, which in all cases co-chromatographed with standard BP, represented 1 – 65% of the total in the bladder medium and 1 – 33% of the total in the medium from the bronchus. The amount of polar metabolite(s) formed was inversely correlated with the amount of reisolated BP in both the bladder ($r = -0.95$) and the bronchus ($r = -0.95$). Neither one of these parameters was correlated with time elapsed after death until culture of the tissue ($0 < r < 0.21$); or with extent of covalent binding ($-0.19 < r < 0$). The identified metabolites of BP never represented >3% of the total amount applied to the column (data not shown).

Discussion

Harris and co-workers (2,3,6 – 8) investigated the metabolism of BP in human bronchial explants obtained from patients either at surgery or "immediate" (tissues removed within 0.5 – 1 h after death) autopsy. In the present study, all bronchial specimens were obtained at autopsy after periods of up to 6 h following death. Our results confirm the reports of Harris, *et al.* on the metabolism and DNA binding of BP by cultured human bronchus and demonstrate the feasibility of investigating the metabolism of at least one carcinogen, BP, in specimens taken at autopsy as long as 6 h after death. In addition, this is the first report on the metabolism and DNA binding of BP in explants of human bladder.

Explants of human bronchus obtained at autopsy as long as 6 h after death were capable of metabolizing BP into forms that were bound to cell DNA. The average binding levels of BP metabolites to bronchial cell DNA found in this study, [3.1 ± 1.9 μmol BP/mol deoxyribonucleotide (16 cases)] are similar to those found by Harris, *et al.* (2) who observed an average binding level of 1.2 ± 1.2 μmol BP/mol deoxyribonucleotide in bronchial explants cultured from 28 cases. Both studies employed similar conditions for explant culture of bronchus and time of exposure (24 h) of the explants to [^{3}H]BP. However, Harris, *et al.* used a higher concentration of [^{3}H]BP (1.5 μM) in the medium and, isolated the DNA by $CsCl_2$ density gradient centrifugation.

It has been suggested that differences among individuals with respect to their ability to metabolize carcinogens into forms that bind to DNA could be associated with carcinogenic risk (3). In the present study, there was a 10-fold variation among individuals in the binding levels of BP metabolites to bronchial cell DNA (0.6 – 6.0 μmol BP/mol deoxyribonucleotide). This is a smaller range of variation than that reported by Harris, *et al.* (3) who found a 75-fold variation in BP binding to bronchial DNA. The smaller variation observed in the present study may be due to the fact that all tissues were taken at autopsy whereas, Harris, *et al.*, obtained tissues from both surgery and "immediate autopsy". In addition, fewer cases were used in the present study. The maximum binding levels observed in the two studies were similar; in the present study, 6.0 μmol BP/mol deoxyribonucleotide versus 4.9 μmol BP/mol deoxyribonucleotide in the study of Harris, *et al.* (3). Histological studies indicated that cultured bronchial and bladder specimens from some cases were more healthy than others. Therefore, it is possible that at least some of the interindividual variation in carcinogen – DNA binding levels may have been due to differences in cellular physiology and viability.

There are no reports on the ability of human bladder epithelium to metabolize chemical carcinogens, including BP. Yet, as mentioned above, epidemiological studies indicate an increased relative risk of bladder cancer among cigarette smokers (21). In addition, it has been known for some time that bladder cancer is associated with exposure to chemical carcinogens in the work place, including aromatic amines (32). Perhaps, one reason that the bladder has not been used for metabolic studies of chemical carcinogens is the assumption that the metabolic activation of bladder carcinogens occurs predominately in the liver (33), followed by their conjugation and excretion into the urine. These metabolites become covalently bound to urothelial cell DNA after either

Table I

Metabolism and covalent binding of [^{3}H]BP by explants of human bladder and bronchus

Case No. (sex, age)	Time after death until tissue cultured (h)	Bladder			Bronchus		
		Metabolism[a]			Metabolism[a]		
		polar fraction	BP fraction	covalent[b] binding	polar fraction	BP fraction	covalent[b] binding
23 (M,29)	6	69.5	5.4	0.5	–	–	4.1
35 (M,37)	5	76.0	13.1	1.0	73.0	10.9	0.6
34 (F, 42)	3.5	80.4	10.4	1.6	70.9	15.4	2.5
31 (M,55)	5	78.1	1.4	1.7	83.1	1.2	1.1
77 (M,63)	4.5	–	–	2.0	–	–	2.1
73 (M,76)	5	29.7	65.3	2.7	46.6	31.1	0.6
33 (M,75)	2.5	64.1	27.0	2.9	58.9	32.8	1.4
9 (F, 22)	2.5	–	–	4.4	–	–	5.8
22 (M,33)	4	80.2	2.2	8.3	72.8	10.7	2.1
14 (F, 55)	2.5	79.9	1.2	8.4	72.4	4.6	5.2
15 (M,10)	3	80.9	1.5	8.7	72.7	6.6	1.9
103 (M,67)	3	–	–	8.8	–	–	5.0
5 (M,15)	6	–	–	10.4	–	–	6.0
100 (M,25)	3	36.3	40.2	10.8	35.4	43.3	2.4
19 (F, 50)	2.5	85.1	1.0	13.1	75.3	2.0	3.8
24 (M,55)	3	84.9	1.0	17.1	81.6	1.5	5.7

[a]A 0.9 ml portion of the medium was mixed with 0.1 ml of methanol and chromatographed as described under **Materials and Methods**. Fractions were collected until the elution of [^{3}H]BP was complete. Numbers represent percentages of the total amount of radioactive material chromatographed; –, sample not analyzed.
[b]μmol BP/mol deoxyribonucleotide.

enzymatic deconjugation by the bladder epithelium itself or by non-enzymatic hydrolysis (34,35). However, the present experiments demonstrate that human bladder epithelium itself can enzymatically activate BP into metabolites that bind to DNA. The BP–DNA adducts formed in human bladder epithelium are chromatographically identical to those formed in other human tissues including the bronchus (8,19). Therefore, it can be assumed that the bladder is fully capable of metabolizing BP and, potentially, other carcinogens which may be eliminated as such, bound to DNA, or become conjugated.

In 12 of the 16 cases used in this study, the level of binding of BP metabolites to DNA in explants of cultured human bladder was higher than that in the bronchus from the same individual. This suggests that human bladder has a greater potential to metabolize BP than human bronchus since direct comparisons on the same individual would tend to eliminate genetic factors that could contribute to variability in metabolism. Therefore, it is possible that the human bladder may be susceptible to carcinogenic transformation following exposure to BP or other polycyclic hydrocarbons.

As reported previously by Jeffrey, *et al.* (19) for explants of human and bovine bronchus, Autrup, *et al.* (9) for human bronchus and rodent trachea, and Autrup, *et al.* (11) for explants of human colon, the major BP–DNA adduct observed in the present study for both human bladder and bronchus co-chromatographed (Figure 4) with the 7R-*anti* diol-epoxide deoxyguanosine (dG) standard (7R-*anti* diol-epoxide-dG). One of the two minor adducts co-chromatographed with standard 7S-*anti* diol-epoxide-dG, while the other minor adduct, eluting ahead of 7S-*anti* diol-epoxide-dG, chromatographed in a manner similar to the "unknown II" adduct reported previously in human and animal respiratory tissues by Autrup, *et al.* (8). Osborne, *et al.* (36) demonstrated that reaction of the (±) *anti* diol-epoxide with DNA produces minor, albeit unstable, adducts at the guanine O^6 and N7 positions. On the other hand, one investigation has shown that BP–deoxycytidine adducts may elute in this region of the chromatogram (37). There is no evidence of adducts to deoxyadenosine in these tissues since they would be expected to elute after the dG adducts (38). There are two reports of *syn* diol-epoxide dG adducts formed by explants of human tissue (19,38); however, *syn* diol-epoxide standards were not used in the present study.

An unexpected result was the observation that the majority of the radioactive material in the medium following incubation of human bladder and bronchus explants with [^{3}H]BP was either unmetabolized BP or present in a highly polar fraction, and that very little (<3%) of the total amount chromatographed, eluted with the commonly known unconjugated metabolites of BP. In order to quantitate the unconjugated metabolites, it was necessary to pool media and prepare an ethyl acetate extract. These studies are now in progress. The amount of radioactivity in the highly polar fraction was inversely correlated with the quantity of unmetabolized BP (Table I). Preliminary results have also revealed that, in most cases, the majority of this polar material cannot be extracted with organic solvents and is refractory to hydrolysis by β-glucuronidase and aryl sulfatase, possibly indicating the presence of glutathione conjugates or other conjugated or unconjugated polar material. It will be of interest to determine if this highly polar material is similar to that demonstrated by Boroujerdi, *et al.* (39) in the pre-9,10-dihydrodiol region of their h.p.l.c. chromatograms of rat biliary excretions following i.v. injection of [^{3}G-H]BP.

Similar to the DNA-binding data, large interindividual differences exist in the extent of BP metabolism (Table I). Part of this variation is undoubtedly due to differences in the amount of tissue incubated per unit volume of medium and, to some extent, the relative viability of the explants.

In summary, explants of human bladder and bronchus were fully capable of metabolizing the carcinogen, BP, into forms that were bound covalently to cellular DNA. When compared to other cultured human tissues, the bladder appears to have a greater ability to metabolize BP into forms that bind to DNA than any other human tissue yet examined. However, this ability is not significantly different ($p < 0.05$, t-test) from that of bronchus, and a larger number of tissues will need to be analyzed before a definite statement can be made. In an earlier report, Autrup, *et al.* (40) summarized comparative studies on BP metabolism in explants of different human tissues. On the basis of this summary, and the results of the present study, we may rank human tissue with respect to its ability to bind metabolized BP as follows: bladder ≥ bronchus > trachea = esophagus > colon.

Acknowledgments

We would like to thank Drs. James Patrick, Majid Alousi and Amira Gohara for assistance in the collection of human tissues. In addition, the excellent technical assistance of Merrill Babcock and Gail Cothern and secretarial assistance of Bonnie Greener is appreciated. This work was supported by Environmental Protection Agency Grant #807670.

References

1. Higginson,J., and Muir,C. (1973), Epidemiology of cancer, in Holland, J. and Frei,E. (eds.), *Cancer Medicine,* Lea and Febiger, pp. 241-306.
2. Harris,C.C., Frank,A.L., van Haaften,C., Kaufman,D.G., Connor,R., Jackson,F., Barrett,L.A., McDowell,E.M., and Trump,B.F. (1976), Binding of [^{3}H]benzo[a]pyrene to DNA in cultured human bronchus, *Cancer Res.*, **36**, 1011-1018.
3. Harris,C.C., Autrup,H., Connor,R., Barrett,L.A., McDowell,E.M., and Trump,B.F. (1976), Interindividual variation in binding of benzo[a]pyrene to DNA in cultured human bronchi, *Science (Wash.),* **194**, 1067-1069.
4. Harris,C.C., Autrup,H., Stoner,G.D., McDowell,E.M., Trump,B.F. and Schafer,P. (1977), Metabolism of dimethylnitrosamine and 1,2-dimethylhydrazine in cultured human bronchi, *Cancer Res.*, **37**, 2309-2311.
5. Harris,C.C., Autrup,H., Stoner,G.D., McDowell,E.M., Trump,B.F. and Schafter,P. (1977), Metabolism of acyclic and cyclic N-nitrosamines in cultured human bronchi, *J. Natl. Cancer Inst.*, **59**, 1401-1406.
6. Harris,C.C., Autrup,H., Stoner,G., Yang,S.K., Lentz,J.C., Gelboin,H.V., Selkirk,J.K., Connor,R.J., Barrett,L.A., Jones,R.T., McDowell,E.M., and Trump,B.F. (1977), Metabolism of benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene in cultured human tissues: bronchus and pancreatic duct, *Cancer Res.*, **37**, 3349-3355.
7. Autrup,H., Harris,C.C., Stoner,G.D., Selkirk,J.K., Schafer,P.W., and Trump,B.F. (1978), Metabolism of [^{3}H]-benzo[a]pyrene by cultured human bronchus and cultured human pulmonary alveolar macrophages, *Lab. Invest.*, **38**, 217-224.
8. Autrup,H., Wefald,F.C., Jeffrey,A.M., Tate,H., Schwartz,R.D., Trump,B.F., and Harris,C.C. (1980), Metabolism of benzo[a]pyrene by cultured tracheobronchial tissues from mice, rats, hamsters, bovines and humans, *Int. J. Cancer,* **25**, 293-300.
9. Autrup,H., Harris,C.C., Stoner,G.D., Jesudason,M.L., and Trump, B.F. (1977), Binding of chemical carcinogens to macromolecules in cultured human colon, *J. Natl. Cancer Inst.*, **59**, 351-354.
10. Autrup,H., Harris,C.C., Schwartz,R.D., Trump,B.F., and Smith,L. (1980), Metabolism of 1,2-dimethylhydrazine by cultured human colon, *Carcinogenesis,* **1**, 375-380.
11. Autrup,H., Harris,C.C., Trump,B.F., and Jeffrey,A.M. (1978), Metabolism of benzo[a]pyrene and identification of the major benzo[a]pyrene–DNA adducts in cultured human colon, *Cancer Res.*, **38**, 3689-3696.
12. Cohen,G.M., Haws,S.M., Moore,B.P., and Bridges,J.W. (1976), Benzo[a]pyren-3-yl hydrogen sulphate, a major ethyl acetate-extractable metabolite of benzo[a]pyrene in human, hamster and rat lung cultures, *Biochem. Pharmacol.*, **25**, 2561-2570.
13. Stoner,G.D., Harris,C.C., Autrup,H., Trump,B.F., Kingsbury,E.W., and Myers,G.A. (1978), Explant culture of human peripheral lung. I. Metabolism of benzo[a]pyrene, *Lab. Invest.*, **38**, 685-692.
14. Harris,C.C., Autrup,H., Stoner,G.D., Trump,B.F., Hillman,E., Schafer,P.W., and Jeffrey,A.M. (1979), Metabolism of benzo[a]pyrene, N-nitrosodimethylamine, and N-nitrosopyrrolidine and identification of the major carcinogen-DNA adducts formed in cultured human esophagus, *Cancer Res.*, **39**, 4401-4406.
15. Grover,P.L., MacNicoll,A.D., Sims,P., Easty,G.C., and Neville,A.M., (1980), Polycyclic hydrocarbon activation and metabolism in epithelial cell aggregates prepared from human mammary tissue, *Int. J. Cancer,* **26**, 467-475.
16. Mass,M.J., Rodgers,N.T., and Kaufman,D.G. (1981), Benzo[a]pyrene metabolism in organ cultures of human endometrium, *Chem.-Biol. Interactions,* **33**, 195-205.
17. Kuroki,T., Nemoto,N., and Kitano,Y. (1980), Metabolism of benzo[a]pyrene in human epidermal keratinocytes in culture, *Carcinogenesis,* **1**, 559-565.
18. Lake,R.L., Pezzutti,M.R., Kropko,M.L., Freeman,A.E., and Igel,H.J. (1977), Measurement of benzo[a]pyrene metabolism in human monocytes, *Cancer Res.*, **37**, 2530-2537.
19. Jeffrey,A.M., Weinstein,I.B., Jennette,K.W., Grzeskowiak,K., Nakanishi,K., Harvey,R.G., Autrup,H., and Harris,C. (1977), Structures of benzo[a]pyrene-nucleic acid adducts formed in human and bovine bronchial explants, *Nature,* **269**, 348-350.
20. U.S. Public Health Service. The health consequences of smoking. U.S. Dept. of Health, Education and Welfare, Public Health Service, Washington, DC:DHEW Publ. No. (HSM) 71-7513, (1971); (HSM) 73-8704, (1974); and (CDS) 76-8704, (1976).
21. Cole,P., Monson,R.R., Haring,H., and Friedell,G.M. (1971), Smoking and cancer of the lower urinary tract, *N. Engl. J. Med.*, **284**, 129-134.
22. Wynder,E., and Hoffman,D. (1968), Experimental tobacco carcinogenesis, *Science (Wash.),* **162**, 862-871.
23. Daniel,F.B., Stoner,G.D., Schenck,K.M., Schut,H.A.J., and Skeel,R.T. (1981), Metabolism and DNA binding of chemical carcinogens in cultured human bronchus and bladder, *Proc. Am. Assoc. Cancer Res. Am. Soc. Clin. Oncol.*, **22**, 100.
24. McDowell,E.M., and Trump,B.F. (1976), Histologic fixatives suitable for diagnostic light and electron microscopy, *Arch. Pathol. Lab. Med.*, **100**, 405-414.
25. Trump,B.F., McDowell,E.M., Barrett,L.A., Frank,A.L., and Harris,C.C. (1974), Studies of ultrastructure, cytochemistry, and organ culture of human bronchial epithelium, in Karbe,E., and Park,J. (eds.), *Experimental Lung Cancer,* New York: Springer-Verlag, Inc., pp. 548-563.
26. Lechner,J.F., Babcock,M.S., Marnell,M., Narayan,K.S., and Kaighn,M.E. (1980), Normal human prostate epithelial cultures, in Harris,C.C., Trump,B.F., and Stoner,G.D., (eds.). *Methods and Perspectives in Cell Biology,* Vol. 2, Academic Press, New York, pp. 195-225.
27. Remsen,J., Jerina,D., Yagi,I.T., and Cerutti,P. (1977), *In vitro* reaction of radioactive 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo-[a]pyrene and 7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]-pyrene with DNA, *Biochem. Biophys. Res. Commun.*, **74**, 934-940.
28. Kirby,K.S. (1965), Isolation and characterization of ribosomal ribonucleic acid, *Biochem. J.*, **96**, 266-269.
29. Kissane,J.M., and Robbins,E. (1958), The fluorometric measurement of DNA in animal tissues with special reference to the central nervous system, *J. Biol. Chem.*, **233**, 184-188.
30. Stoner,G.D., Harris,C.C., Myers,G.A., Trump,B.F., and Connor,R.D. (1980), Putrescine stimulates growth of human bronchial epithelial cells in primary culture, *In Vitro,* **16**, 399-406.
31. Stoner,G.D., Katoh,Y., Foidart,J.-M., Myers,G.A., and Harris,C.C. (1980), Identification and culture of human bronchial epithelial cells, in Harris,C.C., Trump,B.F., and Stoner,G.D. (eds.), *Methods in Cell Biology,* Vol. 21A, Academic Press, New York, pp. 15-35.
32. Case,R.A.M., Hosker,M.E., McDonald,D.B., and Pearson,J.T. (1954), Tumors of the urinary bladder in workmen engaged in the manufacture and use of certain dyestuff intermediates in the British chemical industry. 1. The role of aniline, benzidine, alpha-naphthylamine and beta-naphthylamine, *Br. J. Ind. Med.*, **11**, 74-104.
33. Kadlubar,F.F., Miller,J.A., and Miller,E.C. (1977), Hepatic microsomal N-glucuronidation and nucleic acid binding of N-hydroxy arylamines in relation to urinary bladder carcinogenesis, *Cancer Res.*, **37**, 805-814.
34. Mochizuki,M., Irving,C.C., Anjo,T., Wakabayashi,Y., Suzuki,E., and Okada,M. (1980), Synthesis and mutagenicity of 4-(N-butylnitrosamine)-4-hydroxybutyric acid lactone, a possible activated metabolite of the proximate bladder carcinogen N-butyl-N-(3-carboxypropyl) nitrosamine, *Cancer Res.*, **40**, 162-165.
35. Kadlubar,F.F., Unruh,L.E., Beland,F.A., Straub,K.M., and Evans,F.E. (1980), *In vitro* reaction of the carcinogen, N-hydroxy-2-naphthylamine, with DNA at the C-8 and N^2 atoms of guanine and at the N^6 atom of adenine, *Carcinogenesis,* **1**, 139-150.
36. Osborne,M.R., Jacobs,S., Harvey,R.G., and Brookes,P. (1981), Minor products from the reaction of (+) and (−) benzo[a]pyrene-anti-diolepoxide

with DNA, *Carcinogenesis*, **2**, 553-558.
37. Meehan,T., Straub,K., and Calvin,M. (1977), Benzo[a]pyrene diol epoxide covalently binds to deoxyguanosine and deoxyadenosine in DNA, *Nature*, **269**, 725-727.
38. Autrup,H., Schwartz,R.D., Essigmann,J.M., Smith,L., Trump,B.F., and Harris,C.C. (1980), Metabolism of aflatoxin B_1, benzo[a]pyrene, and 1,2 dimethylhydrazine by cultured rat and human colon, *Teratogenesis, Carcinogenesis and Mutagenesis*, **1**, 3-13.
39. Boroujerdi,M., Kung,H., Wilson,A., and Anderson,M. (1981), Metabolism and DNA binding of benzo[a]pyrene *in vivo* in the rat, *Cancer Res.*, **41**, 951-957.
40. Autrup,H., Jeffrey,A.M., and Harris,C.C. (1980), Metabolism of benzo[a]pyrene by cultured human tissues, in Bjorseth,A., and Dennis,A.J. (eds.), *Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects*, Battelle Press, pp. 89-106.